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(71) Applicant: **SCHERING CORPORATION**  
2000 Galloping Hill Road  
Kenilworth, New Jersey 07033(US)

(72) Inventor: **Lydon, Nicholas**  
Bollwerkstrasse 30C  
CH-4102 Binningen(CH)

(72) Inventor: **Favre, Catherine**  
2 Cours Gambetta  
F-69007 Lyon(FR)

(72) Inventor: **Bove, Sylvie**  
11, avenue de Bâle  
F-68330 Huningue(FR)

(74) Representative: **Ritter, Stephen David et al,**  
Mathys & Squire 10 Fleet Street  
London EC4Y 1AY(GB)

(54) **Monoclonal antibodies to interferon alpha 2 and hybridomas producing such antibodies.**

(57) This invention relates to monoclonal antibodies to interferon alpha<sub>2</sub> and to hybridomas producing such antibodies. These antibodies are preferably of the immunoglobulin subclass IgG1 and preferably do not bind to alpha<sub>1</sub> of alpha<sub>2</sub> interferon.

They can be used in assaying, purifying or isolating proteins, such as interferon alpha<sub>2</sub>, to which they are specific.

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MONOCLONAL ANTIBODIES TO INTERFERON  $\alpha_2$  AND  
HYBRIDOMAS PRODUCING SUCH ANTIBODIES

This invention relates to novel monoclonal antibodies to interferon  $\alpha_2$  and to hybridomas producing such antibodies.

- 5 It is well known in the art that it is possible to obtain a cell line which is able to produce a homogenous, i.e. monoclonal, antibody. The basis technique was originally described by Kohler and Milstein [Nature 256, (1975)] and comprises the fusion of mouse myeloma cells  
10 to spleen cells and selection of clones capable of producing the desired antibody. This general procedure has also been described in U.S. Patents 4.364.932, 4.364.934, 4.364.935, 4.364.937 and 4.361.550.

Although the general method has been known for some years, there are in fact numerous difficulties to overcome and specific variations have to be found for each case. There is no certainty that a suitable hybridoma  
5 will be found and, equally, there is no certainty that the hybridoma will produce an antibody having the desired properties.

As is well known, antibodies are useful for various purposes. Thus monoclonal antibodies may in particular  
10 be useful for assaying (diagnostic kit) or for isolation and purification of the proteins for which they are specific. (See for example PCT/GB 81/00239, Publication No. W082/01773).

The PCT patent application number PCT/GB88/00067 as well  
15 as "J. gen. Virol. (1981) 53, 257-265" describe a monoclonal antibody, NK2, which is specific to  $\alpha$ -interferons in general and does not bind to other interferons, e.g.  $\beta$ -interferon etc. The term  $\alpha$ -interferon or leukocyte  
interferon, however, covers a group of 15 or more distinct  
20 molecular entities and the antibodies of this invention are clearly different from NK2 in that they are of different Ig subclass and will bind to different  $\alpha$ -interferon entities.

More recent research has made it possible to isolate  
25 single species from the group of  $\alpha$ -interferons, to produce each species in larger quantities and to purify it to a homogenous compound.

One of these  $\alpha$ -interferons called interferon  $\alpha_2$  (IFN $\alpha_2$ ) has in various tests proved to be a most promising pharmaceutically active substance useful for combatting various diseases. IFN $\alpha_2$  is widely described in literature and may be obtained either by isolation from blood samples or by so-called recombinant DNA technology, e.g. as described in the published European Patent Application No. 0032134.

It is obvious that it is highly desirable to have antibodies which are specific to IFN $\alpha_2$  only and which do not bind to other closely related  $\alpha$ -interferons or to  $\beta$ -interferon etc.

The purpose of this invention therefore was to isolate monoclonal hybridomas which are able to produce monoclonal antibodies binding strongly to IFN $\alpha_2$  and not or only weakly to other  $\alpha$ -interferons.

As mentioned above, such antibodies are highly useful for various purposes, e.g. for assaying or purifying (affirmative chromatography) IFN $\alpha_2$ . A further possible use is for isolation of shorter interferon  $\alpha_2$ -type protein molecules, e.g. portions of IFN $\alpha_2$ , which, though smaller than the original IFN $\alpha_2$ -molecule, still have the same or a similar characteristics and activities as IFN $\alpha_2$  for a particular molecular structure and, accordingly, respond to invasions of foreign molecules into the mammalian body.

The hybridomas and the antibodies of this invention may be obtained by the following procedure:

1. Mice are immunized with several injections of  $\text{IFN}\alpha_2$ . The type of mice used is not critical but good results are achieved with Balb/c females. The antigen ( $\text{IFN}\alpha_2$ ) may be applied in any suitable form, e.g. in complete Freund Adjuvant (CFA) emulsified with phosphate buffered saline (PBS) (ratio 1:1). The number of injections and the quantity of antigen administered must be such, that useful quantities of suitably primed splenocytes are produced. Usually, immunisation consists of three intraperitoneal injections with  $10\mu\text{g}$  of antigen at about 2-week intervals. This is followed by a further boost consisting of  $10\mu\text{g}$  antigen in PBS Intravenously and  $10\mu\text{g}$  antigen in CFA/PBS Intraperitoneally.
2. The spleens of the immunized mice are removed and spleen suspensions are prepared. This procedure follows well known techniques.
3. The spleen cells are fused with mouse myeloma cells. The technique for fusing myeloma cells with spleen cells is well known. Most preferably the fusion is achieved by heating a mixture of the two cell types with certain chemical ingredients (fusion promoter), e.g. polyethyleneglycol (PEG) having an average molecular weight from about 1000 to 4000 (PEG1000). Several mouse myeloma cell lines are known and easily available. Preferred are cell lines which are HGPRT-deficient (HGPRT = Hypoxanthine Guanosyl Phosphoribosyl Transferase) and accordingly will not survive in HAT (culture medium comprising hypoxanthine, aminopterin and thymidine). Preferably the myeloma cell line used should be of the non-secreting type in that it does not itself produce any antibody. A suitable cell line for the

purpose of this invention is the so-called NS1 cell line. These cells were derived from P3/X63-A8 myeloma cells by Köhler and Milstein.

4. The fused spleen cells are cultured in several  
5 separate containers. Also this step follows standard procedures. The cell cultures obtained in step 3 are mixtures of fused spleen cells, unfused spleen cells and unfused myeloma cells. Preferably the cultivation is carried out in a medium which will eliminate the unfused  
10 myeloma cell line, e.g. in a HAT medium. Those unfused spleen cells which are non-malignant will normally stop growing after a short period of time, whereas the fused cells, which are HGPRT<sup>+</sup> can grow in HAT medium.
5. The supernatants of the hybridoma cells in each  
15 container are tested for the presence of IFN $\alpha_2$ -antibodies. This test may conveniently be carried out by applying an enzyme linked immunosorbant assay (ELISA). In the present case antibodies linked to the enzyme alkaline phosphatase were chosen, but also other procedures are  
20 conceivable.
6. Hybridomas producing the desired antibodies are selected and cloned. The cloning is preferably carried out using the limiting dilution technique.
7. The desired antibodies are produced by means of  
25 the selected hybridomas. This production may be achieved in vitro by culturing the hybridoma in a suitable medium followed by isolation of the antibody, however, this method may not yield sufficient quantities.

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A preferred method for producing larger quantities of antibody uses an in vivo approach. The hybridoma is injected back into mice where it will cause production of ascites fluid containing substantial quantities of the desired antibody which is then isolated according to standard procedures.

According to the present invention there are provided hybridomas capable of producing antibodies against interferon  $\alpha_2$ ; methods for producing the antibodies, and methods and compositions for utilizing these antibodies.

Although only a relatively small number of hybridomas producing antibodies against  $\alpha_2$  interferon are described, it is contemplated that the present invention encompasses all monoclonal antibodies exhibiting the characteristics described herein.

Further included within the subject invention are methods for preparing the monoclonal antibodies described above employing the hybridoma technique illustrated herein. Although only limited examples of hybridomas are given herein, it is contemplated that one skilled in the art could follow the immunization, fusion, and selection methods provided herein and obtain other hybridomas capable of producing antibodies having the reactivity characteristics described herein. Since the individual hybridoma produced from a known mouse myeloma cell line and spleen cells from a known species of mouse cannot be further identified except by reference to the antibody produced by the hybridoma, it is contemplated that all hybridomas producing antibody having the reactivity characteristics described above are included within the subject invention, as are methods for making this antibody employing the hybridoma.

Further aspects of the invention are methods of treatment or diagnosis employing the monoclonal antibodies exhibiting the pattern of reactivity provided herein.

E X A M P L E1. Immunization of Mice

Balb/C female mice were immunized by three injections at fifteen days intervals with 10  $\mu$ g of IFN $\alpha_2$  (Schering-Plough Corporation) in CFA/PBS emulsion (1:1). A total volume of 0.2 ml was injected intraperitoneally into each mouse. Fifteen days after the third injection a boost was made by injecting 10  $\mu$ g of antigen in CFA intraperitoneally and at the same time 10  $\mu$ g of antigen in PBS intravenously. Four days after the last injection the mice were bled and their spleen excised for fusion.

2. Cell Fusion

The spleen was suspended in PBS (Ca $^{++}$ - and Mg $^{++}$ -free) and a cell count was carried out (one spleen comprises approximately  $10^8$  cells).

After filtration through sterile gauze, the cells were washed twice in cold Ca $^{++}$ - and Mg $^{++}$ -free PBS (GIBCO CAT 420). The mouse myeloma cells (NS1) were washed (3 times with the same type PBS) and the two cell types were mixed and centrifuged together. The mixture comprised  $\sim 10^8$  spleen cells and  $\sim 10^7$  NS1 cells.

About 0.2ml of supernatant was left over the cells. After disrupting the pellet by gentle agitation of the tube, 1ml of PEG 1000 (Merck Art. 9729), 50% in PBS without Ca $^{++}$  and Mg $^{++}$ , was added dropwise during 1 mn with constant agitation at 37°C. After thirty seconds



of agitation at 37°C., the tube was filled slowly with warm PBS without  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  and centrifuged. The cells were then directly re-suspended in HAT Medium and distributed into 24 well plates (1ml per well with about  
5  $2 \times 10^6$  cells per well). At this stage, non-treated splenocytes (1:10 of each spleen) were added as feeder cells.

### 3. Culture of the Hybridoma Cells

24 hours after the fusion, 1ml of HAT medium was added to  
10 each well. Fresh medium was added three times a week to all the wells. The selection was achieved by culturing the hybridomas in HAT Medium during 3 weeks followed by culturing the cells during three subsequent weeks in HT medium (same medium but without aminopterin) and  
15 then kept in normal culture medium (RPMi 1640 with 10% FCS).

As soon as possible, the hybridoma cells were frozen using standard techniques. The supernatant was kept and tested for the presence of anti-IFN $\alpha_2$  antibodies.

### 20 4. Test (screening) For Presence Of Anti-IFN $\alpha_2$ Antibodies

The presence of anti-IFN $\alpha_2$  antibodies in the supernatants of the hybridoma cell cultures was tested by an enzyme linked immunosorbant assay (ELISA). The use of anti-  
25 bodies linked to the enzyme alkaline phosphatase following classical techniques (see "Enzyme Linked Immunosorbant Assay"; A. Voller, D. Bidwelland, A. Bartlett; Manual of Clinical Immunology, chapter 45, p. 359) was chosen and

adapted for detection of monoclonal anti-IFN $\alpha_2$  antibodies.  
The test used comprised the following steps:

- 5           1) Coating of the plate with 100 ng IFN $\alpha_2$  per  
            well (dilution 0.5  $\mu$ g/ml in coating buffer;  
            0.2 ml per well)  
            ↓  
            overnight 4°C.  
            ↓  
            flick over the sink  
            ↓  
10          2) + 0.2 ml RPMI-FCS per well  
            ↓  
            1h incubation at room temperature  
            ↓  
            4 washes PBS-Tween  
            ↓  
            3) 0.2 ml of hybridoma supernatant per well  
            ↓  
            2h incubation room temperature  
            ↓  
            4 washes PBS-Tween  
            ↓  
15          4) 0.2 ml anti-mouse Ig-AP 1:500 in RPMI-FCS  
            ↓  
            2h incubation room temperature  
            ↓  
            4 washes PBS-Tween  
            ↓  
            5) 0.2 ml PNPP in diethanolamine buffer  
            ↓  
            readings after 15mn, 30mn, 45mn, 1h.

The absorption of  $\text{IFN}\alpha_2$  to the bottom of the plates (96 well plates; NUNC Immune plate I CAT No. 2-39454) was performed in coating buffer (carbonate-bicarbonate buffer) containing 1.59 g  $\text{Na}_2\text{CO}_3$ , 2.93 g  $\text{Na HCO}_3$ , 0.2 g  $\text{NaN}_3$  per liter of distilled water. After one night at 4°C., the wells were saturated with protein by an incubation of 1 hour at room temperature with the culture medium containing RPMI-10% FCS (0.2 ml per well).

The plate was then washed four times with PBS-Tween containing 8g  $\text{NaCl}$ , 0.2 g  $\text{KH}_2\text{PO}_4$ , 2.9 g  $\text{Na}_2\text{HPO}_4$  (12  $\text{H}_2\text{O}$ ), 0.2 g  $\text{KCl}$ , 0.5 ml Tween 20 in one liter distilled water (pH 7.4).

After incubation of the plate with hybridoma supernatants, the presence of mouse anti- $\text{IFN}\alpha_2$  antibodies was revealed by sheep anti-mouse immunoglobulins conjugated with alkaline phosphatase (e.g. NEI-500 from NEN).

After two hours of incubation with the conjugate and four subsequent washings, 0.2 ml of substrate solution was added. The substrate (paranitrophenyl phosphate; PNPP Sigma 104 phosphatase substrate ref.: 104-105) was dissolved (1 tablet of 5 mg for 5 ml of buffer) in a buffer containing 100 mg of  $\text{MgCl}_2 \cdot (6\text{H}_2\text{O})$ ; 0.2 g  $\text{NaN}_3$ , 97 ml diethanolamine in one liter distilled water (pH 9.8, adjusted with  $\text{HCl}$ ). The optical density at 405 nm was read at different time intervals after addition of substrate (Autoreader MR580 from Dynatech). Strong positive hybridomas were selected for cloning. These were designated 6N5, 7N2 and 7N4.

5. Cloning

The cloning of the hybridoma cells was performed by the limiting dilution technique.

Hybrid cells were diluted in the culture medium and distributed into 96 well plates (flat bottom linbro 76003-05) in order to have 60 cells per plate (0.2 ml per well). Peritoneal macrophages of Balb/C mice were used as feeder cells; these were collected by washing the peritoneal cavity of mice with HBSS (GIBCO Cat. No. 406) containing 1% of antibiotics (Penicilline-Streptomycine) at 4°C. Usually the peritoneal macrophages recovered from one mouse were sufficient for one 96 well plate (about 2 to 4 x 10<sup>5</sup> cells per well).

After about three weeks, the clones could be seen by eye. They were then transferred to 24 well plates. At this stage the clones were frozen as quickly as possible. The supernatants were then kept and tested for anti-IFN $\alpha_2$  activity.

The hybridoma 6N5 gave one active clone which designated 6N5-2-I. Hybridoma 7N2 gave one active clone which was designated 7N2-4. Hybridoma 7N4 produced one active clone which was designated 7N4-1. The monoclonal antibodies produced by the active hybridomas were designated A6N5-2-I, A7N2-4 and A7N4-1. The monoclonal hybridomas were deposited in the "COLLECTION NATIONALE DE CULTURE DE MICROORGANISMES" at "INSTITUT PASTEUR", France on February 22, 1984 where they received the deposition numbers I-279 (6N5-2-I), I-278 (7N2-4) and I-277 (7N4-1).

## 6. Production of Ascites Fluid

In order to obtain large amounts of monoclonal antibodies, ascites fluid was induced in Balb/C mice by injecting hybridoma cells.

- 5 Four days before injection of the cells the mice were treated i.p. with 0.5 ml of pristane (2,6,10,14-Tetramethyl-penta-decane Aldrich T22802). After three washes of hybridoma cells with PBS Dulbecco (GIBCO 041.4040), the cell suspension was adjusted to  $2.5 \times 10^7$  cells per  
10 ml and 0.2 ml injected into each mouse ( $5 \times 10^6$  cells per mouse). After a period ranging from ten to twenty days, the ascitic fluid could be collected. After a period of a few days of rest it was possible to collect ascitic fluid from the same mice again. At least two or  
15 three samples of ascitic fluid were harvested from each mouse.

## 7. Isolation and Purification Of the Clones From Ascites Fluids

- Ammonium sulfate precipitation : 27 ml of ascites fluid  
20 was diluted four-fold in cold PBS and placed on ice. An equal volume of saturated  $(\text{NH}_4)_2\text{SO}_4$  solution (4°C) was added slowly with stirring over a period of several minutes: final  $(\text{NH}_4)_2\text{SO}_4$  concentration was 50% saturation. The solution was left on ice for 30 min. Centrifugation  
25 was at  $5000 \times g$  for 10-15 mn. The pellet was recovered and dissolved in 15 ml of buffer containing 40mM-NaCl, 20mM-Tris/HCl, pH 7.8 (buffer A). The re-suspended pellet was dialysed against 100 volumes of buffer containing 20mM-NaCl, 20mM-Tris/HCl, pH 7.8 (buffer B). Prior

to ion exchange chromatography denatured protein was removed by centrifugation at 15,000 x g for 10 min.

DEAE cellulose chromatography : DE 52 (Whatman), which had been equilibrated in buffer B, was packed into a column (2.5 cm x 27 cm) giving a packed bed volume of 132 ml. Packing was carried out with a pumped flow rate of 45 ml/hr/cm. Chromatography was performed at room temperature. Immediately prior to loading, the dialysed sample was adjusted so as to constitute the ionic conditions of buffer B. The sample was loaded at a flow rate of 2ml/min. After washing with buffer B (1/10 of bed volume) the column was eluted with a linear gradient of NaCl (40 mM-200 mM). The total gradient volume was 1 liter. The elution flow rate was 50 ml/hr. 10 ml fractions of eluate were collected.

#### 8. Lyophilisation

Eluate fractions were dialysed against 1% (w/v)  $\text{NH}_4\text{HCO}_3$  for 48 hrs. The final volume following dialysis was 168 ml. The pool was sterilized by filtration through a 0.22 Micron membrane filter (Falcon). 10 ml aliquots of filtrate were transferred into sterile bottles and lyophilised. Sterile conditions were maintained following lyophilisation by using an automatic capping device.

CHARACTERIZATION OF THE 5 ANTI-INTERFERON $\alpha_2$   
ANTIBODIES A6N5-2-I, A6N5-2-II, A7N2-4  
and A7N4-1

5 The characterization of a monoclonal antibody should  
provide the following information:

- (i). Determination of the specificity, i.e. to  
which type of interferons will the antibody  
bind.
- (ii). Determination of Ig subclass.
- 10 (iii). Determination of the effect the antibody has  
on biological functions of the molecule  
(in this case interferons).

1. Specificity of the monoclonal antibodies

15 The specificity was determined by using the ELISA test  
described under item 4 of the Example above.

2. Determination of Ig subclass

The isotype of the monoclonal antibodies purified from  
ascites fluid was tested by an indirect ELISA test.  
The plates were coated with 100ng IFN $\alpha_2$  per well and  
20 treated with RPMI-10% FCS as already described. The  
monoclonal antibodies diluted in RPMI-10% were allowed  
to fix to the antigen coated at the bottom during 2h  
incubation. The wells were then filled with a solution  
of antibodies directed against various mouse Ig sub-  
25 classes (dilution 1:1000). All the anti-isotypes used  
here were produced in rabbits. (IgM; IgG<sub>1</sub>; IgG 2a;

IgG 2b; IgG 3; IGA,  $\lambda$  and K). The presence of anti-isotype antibodies was detected by anti rabbit Ig conjugated to alkaline phosphatase. The conditions of incubation and the reading of the results were as described  
5 for the ELISA test above.

### 3. Functional Tests

#### A. Inhibition of 2'-5' oligo (A) synthetase

Treatment of cells with  $\text{IFN}\alpha_2$  results in the induction of 2'-5' oligo (A) synthetase. This  
10 activity was used as a functional test for  $\text{IFN}\alpha_2$ , i.e. it was determined whether the antibodies inhibit the induction of the 2'-5' oligo (A) synthetase by  $\text{IFN}\alpha_2$ .

In addition to its anti-viral actions,  $\text{IFN}\alpha_2$ -  
15 treatment of cells have been shown to induce various mRNA's and proteins. Among the proteins induced are a number of enzymes, including 2'-5' oligo (A) synthetase. 2'-5' oligo (A) synthetase is activated by dsRNA [e.g. poly (I). (C)] and  
20 produces a heterogeneous family of 2'-5' linked oligoadenylates of which the di-, tri- and tetra-adenylates are the most abundant. Induction of 2'-5' oligo (A) synthetase can be utilized as a reliable marker for the biological activity of  
25 interferons. The Hela  $S_3$  cell line was used to investigate the effects of interferon preparations on 2'-5' oligo (A) synthetase induction. The effect was measured by determining the quantities of 2'-5' oligo (A) produced.



This 2'-5' oligo (A) synthetase test includes the following steps:

- (a) pre-treatment of  $\text{INF}\alpha_2$  with the monoclonal antibody;
  - 5 (b) addition of the immune complex obtained to HeLa  $S_3$  cell cultures; and
  - (c) assaying HeLa  $S_3$  cell extracts from cultures (b) for 2'-5' oligo (A) synthetase activity.
- 10 Step (a): To 5 ml of monoclonal culture supernatant was added 100 ng  $\text{INF}\alpha_2$  per ml. The mixture was subjected to a one hour incubation at 37°C.
- 15 Step (b): 2.5 ml of media obtained according to step (a) were added to HeLa  $S_3$  cell cultures containing 7.5 ml of fresh media (RPMI + 10% CFS), mixed by shaking and incubated for 20 hours at 37°C., in a humidified  $\text{CO}_2$  incubator.
- 20 Step (c): Following washing in PBS, adherent HeLa cells were scraped from plates into 2 ml of PBS (0.01 M-phosphate buffer, pH 7.2, 0.15 M-NaCl). Plates were then washed with an additional 1 ml of PBS. Cells were pelleted at 500 X g for 5 minutes at room temperature. The supernatant liquid was carefully removed using a Pasteur pipette. The pellet was then re-
- 25 suspended in lysis buffer containing 10 mM-KCl, 1.5 mM magnesium acetate, 0.5%-Triton X-100 and 20 mM-Hepes/KOH, pH 7.5. Cells were

lysed by treating at 4°C for 10 minutes. The lysate was immediately centrifuged at 30,000 Xg for 15 minutes. The supernatant material was recovered and is referred to as the HeLa S<sub>3</sub> cell extract. Extracts were subsequently assayed for 2'-5'oligo (A) synthetase activity.

The assays contained, in a final volume of 50  $\mu$ l: 30  $\mu$ l of cell extract, 5mM-ATP, 25mM-Mg (OAC)<sub>2</sub>, 4mM-Fructose 1,6 diphosphate, 1mM-DTT, 100mM-KOAC, 20mM-Hepes/KOH, pH 7.6, 10%-glycerol and 100  $\mu$ g/ml poly (I).(C) (Miles Laboratories). Incubations were at 30°C., for 1 hour and were terminated by heating at 100°C., for 3 minutes. 2'-5' oligo (A) produced was isolated by ion exchange chromatography on DEAE- cellulose. The samples were diluted with 1ml of buffer containing 90mM-KCl, 20mM-Tris-HCl pH 7.6 (start buffer). Diluted samples were passed three times over a column containing 0.5ml of packed DE 52 (Whatman), equilibrated with start buffer. Columns were washed with 10ml of start buffer, then eluted with 1ml of elution buffer (350mM-KCl 20mM-Tris-HCl pH 7.6).

The production of 2'-5' oligo (A) was measured spectrophotometrically at 259nm.

### B. Antiproliferation

A further characteristic of the antibodies is whether they inhibit the antiproliferative effect of IFN $\alpha_2$ . The test was carried out using the Daudi Cell  
5 line (obtained from the American Type Culture Collection) which is extremely sensitive to the antiproliferative action of interferons.

IFN $\alpha_2$  was incubated with monoclonal antibody for 30 mn at 37°C., in a final volume of 50  $\mu$ l. This  
10 was carried out in microtiter culture plates. 100  $\mu$ l of cell suspension containing 10<sup>4</sup> cells was then added to each well. After 3 days incubation in a CO<sub>2</sub> incubator, proliferation was measured by a rapid quantitative colorimetric assay.

15 Three hours before the end of the assay, MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; 10  $\mu$ l of 5 mg/ml solutions) was added to each well. The microtitre culture plate was then incubated for a further 3 hours. Trays were  
20 removed from the incubator and 200  $\mu$ l of 0.04 N HCl in isopropanol was added to each well. After mixing, the trays were read on a Dynatech MR 580 autoreader using a reference wavelength of 630 nm and a test wavelength of 570nm. The blue formazan reaction  
25 product is a quantitative measure of live cell number.

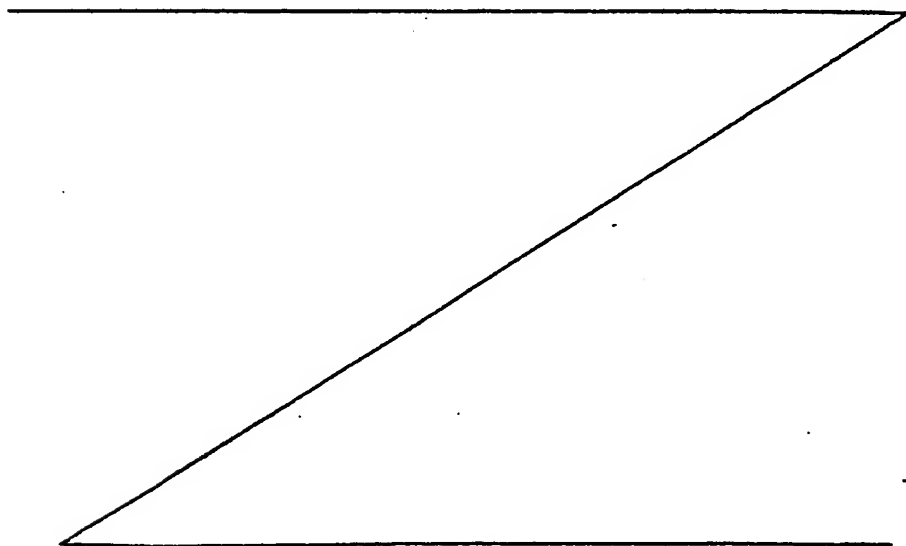
The specificity test as well as the results of tests A and B above with regard to the monoclonal antibodies of this invention and of NK-2 are given in the table below.

	IG-Subclass	Specificity tests					Functional tests			
		$\alpha_2$	$\alpha_1$	$\alpha_7$	$\beta$	30-G	30-E	30-D	2'-5' oligo(A)	Anti-proliferation
Monoclonal										
A6.N5-2-1	IgG1	++	-	-	-	+	-	-	ND	ND
A7N2-4	IgG1	++	-	-	-	+	-	-	no	no
A7N4-1	IgG1	++	-	+	-	-	-	-	yes	yes
-----										
NK-2	IgG2b	++	-	-	-	+	-	-	yes	yes

ND: Not Determined  
 ++: strong positive  
 + : positive  
 - : negative

The Amino acid sequence of Interferon  $\alpha_2$ 

CYS-ASP-LEU-PRO-GLN-THR-HIS-SER-LEU-GLY-SER-ARG-ARG-THR-  
LEU-MET-LEU-LEU-  
ALA-GLN-MET-ARG-ARG-ILE-SER-LEU-PHE-SER-CYS-LEU- LYS-ASP-  
ARG-HIS-ASP-PHE-  
GLY-PHE-PRO-GLN-GLU-GLU-PHE-GLY-ASN-GLN-PHE-GLN-LYS-ALA-  
GLU-THR-ILE-PRO-  
VAL-LEU-HIS-GLU-MET-ILE-GLN-GLN-ILE-PHE-ASN-LEU-PHE-SER-  
THR-LYS-ASP-SER-  
SER-ALA-ALA-TRP-ASP-GLU-THR-LEU-LEU-ASP-LYS-PHE-TYR-THR-  
GLU-LEU-TYR-GLN-  
GLN-LEU-ASN-ASP-LEU-GLU-ALA-CAS-VAL-ILE-GLN-GLY-VAL-GLY-  
VAL-THR-GLU-THR-  
PRO-LEU-MET- LYS-GLU-ASP-SER-ILE-LEU-ALA-VAL-ARG-LYS-TYR-  
PHE-GLN-ARG-ILE-  
THR-LEU-TYR-LEU-LYS-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA-TRP-  
GLU-VAL-VAL-ARG  
ALA-GLU-ILE-MET-ARG-SER-PHE-SER-LEU-SER-THR-ASN-LEU-GLN-  
GLU-SER-LEU-ARG-  
SER-LYS-GLU



Amino acids of the interferon  $\alpha_2$ -fragments 30-G; 30-E and 30-D:

Fragment	Amino acids
30-G	<p>Cys<sup>1</sup>.....Leu<sup>15</sup>-Hser</p> <p>Ile<sup>60</sup>.....Cys<sup>98</sup>.....Leu<sup>110</sup>-Hser</p>
30-E	<p>Arg<sup>22</sup>.....Cys<sup>29</sup>.....Glu<sup>58</sup>-Hser</p> <p>Lys<sup>112</sup>....Cys<sup>138</sup>.....Ile<sup>147</sup>-Hser</p>
30-D	Arg <sup>149</sup> - Glu <sup>165</sup>

As can be seen the antibodies of this invention are all of the Ig subclass IgG1 whereas HK2 is of the subclass IgG2a. The antibodies A6N5-2-I and A7N2-4 do not bind to  $\alpha_1$  and  $\alpha_7$ . All antibodies bind strongly to  $\alpha_2$ . HK2 binds to  $\alpha_2$  and  $\alpha_7$ . None of the antibodies bind to  $\beta$ -interferon.

The substances used in the above tests are either known or their structure is given below. The sequences of  $\alpha_1$  and  $\alpha_7$  have been published, e.g. in:-

Weissmann et al (1982): "Structure and expression of Human Alpha interferon Genes" (Interferons, pp 295, Academic Press);

Scientific American, Vol. 249 No. 2, pp 28-36, (S. Pestka).

C L A I M S

1. Monoclonal  $\alpha$ -interferon antibodies obtainable  
by cultivating monoclonal hybridomas, characterized  
in that the antibodies bind strongly to interferon  
5  $\alpha_2$ .
2. Monoclonal antibodies according to claim 1,  
characterized in that the antibodies are of the  
immunoglobuline subclass IgG1.
3. Antibodies according to claim 2, characterized  
10 in that they
  - (a) bind strongly to interferon  $\alpha_2$ ;
  - (b) do not bind to interferon  $\alpha_1$  or  $\alpha_7$ .
4. Antibody according to claim 3, being the antibody  
designated A6N5-2-I.
5. Antibodies according to claim 2, characterized  
in that they
  - (a) bind strongly to interferon  $\alpha_2$ ;
  - (b) do not bind to interferon  $\alpha_1$  or  $\alpha_7$ ;
  - (c) do not inhibit 2'-5' oligo(A) synthetase  
induction by interferon  $\alpha_2$ ; and
  - (d) do not inhibit the antiproliferative effect  
of interferon  $\alpha_2$ .

6. Antibody according to claim 5, being the anti-body designated A7N2-4.
7. Antibodies according to claim 2, characterized in that they
  - (a) bind strongly to interferon  $\alpha_2$ ;
  - (b) do not bind to interferon  $\alpha_1$ ;
  - (c) inhibit 2'-5' oligo (A) synthetase induction by interferon  $\alpha_2$ ; and
  - (d) inhibit the antiproliferative effect of interferon  $\alpha_2$ .
8. Antibodies according to claim 7 being the anti-body designated A7N4-1.
9. The monoclonal hybridomas designated 6N5-2-I, 7N2-4 and 7N4-1 said hybridomas having being deposited and given the deposit numbers:

6N5-2-I	: I-279
7N2-4	: I-278
7N4-1	: I-277
10. Use of a monoclonal antibody as claimed in any of claims 1 to 8 to assay, purify or isolate a protein, such as interferon alpha<sub>2</sub>, to which it is able to bind.





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0158420

Application number

EP 85 30 1155

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
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Y	--- CHEMICAL ABSTRACTS, vol. 99, no. 1, 4th July 1983, page 380, no. 3950g, Columbus, Ohio, US; A.R.GUENTHER et al.: "Production of monoclonal antibodies to human INF-alpha and their use for analysis of the antigenic composition of various natural interferons" & J. CELL. PHYSIOL 1982 (SUPPL. 2), 61-8 * Abstract *	1,2	TECHNICAL FIELDS SEARCHED (Int. Cl. 4)  A 61 K C 12 P C 12 N
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 20-06-1985	Examiner REMPP G.L.E.
<b>CATEGORY OF CITED DOCUMENTS</b> X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document  T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons  & : member of the same patent family, corresponding document			

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Y	FR-A-2 500 754 (F. HOFFMAN-LA ROCHE) * Page 4, line 1 - page 13, line 17 *	1-9	
Y	THE JOURNAL OF IMMUNOLOGY, vol. 128, no. 6, June 1982, pages 2824-2825, The American Association of Immunologists, US; MITSUNOBU IMAI et al.: "Demonstration of two subtypes of human leukocyte interferon (IFN-alpha) by monoclonal antibodies" * The complete article *	1-9	
Y	NATURE, vol. 296, 15th April 1982, pages 664-665, MacMillan Journal Ltd.; DANIELA MÄNNEL et al.: "A rat monoclonal antibody against mouse alpha and beta interferon of all molecular weight species" * The complete article *	1-9	
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 20-06-1985	Examiner REMPP G.L.E.
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Place of search THE HAGUE		Date of completion of the search 20-06-1985	Examiner REMPP G.L.E.
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